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The Effect of a 20-25 calories % Fat Diet on Plasma Thromboxane B_2 Levels in Human Subjects

Carolyn Wernick

Abstract

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Fourteen males with elevated blood lipids were given a 20-25 Calories % fat diet for 28 days. Fasting blood samples were drawn at zero, two, and four weeks and analyzed for plasma TXB_2 and serum lipids. Plasma TXB_2 was significantly increased the first two weeks with a subsequent decrease the final two weeks. Values were still significantly elevated at four weeks when compared with initial values. Serum cholesterol, HDL cholesterol, and LDL cholesterol were significantly decreased over the four-week period. No significant changes were observed in serum triglycerides.

LOMA LINDA UNIVERSITY

Graduate School

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Each person whose signature appears below certifies that this manuscript in his opinion is adequate, in scope and quality, in lieu of a thesis for the degree Master of Science.

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INTRODUCTION

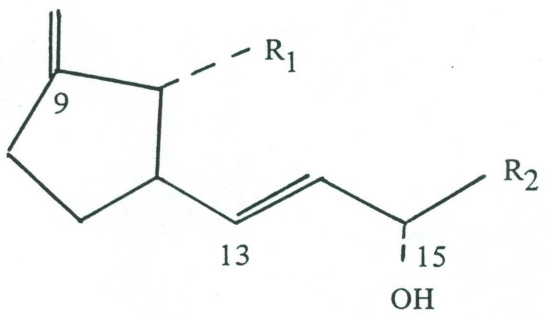
Biosynthesis

Thromboxane A_2 is a prostaglandin identified in human platelets by Svensson, Hamberg, and Samuelsson in 1975 (1, 2). Thromboxane A_2 was first known as a rabbit aorta contracting substance (RCS), a name coined by Piper and Vane (3) because of its ability to contract isolated rabbit aorta. This RCS was found to have two active components, one being more potent than the other. The less active component had a half life of about five minutes and was identified as being the prostaglandin endoperoxides PGG_2 and PGH_2. The more potent substance was an unstable compound with a half life of about 30 seconds. This substance was named thromboxane A_2 and was detected as an unstable intermediate in the conversion of PGG_2 to TXB_2. Thromboxane B_2 is the stable inactive metabolite of TXA_2 (1, 2).

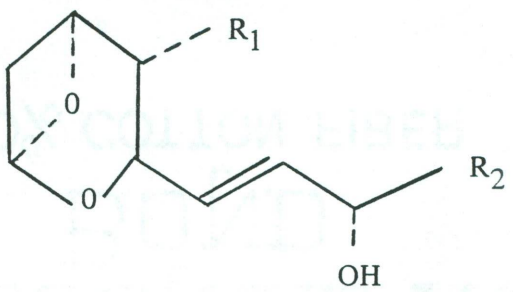
Prostaglandins are synthesized from 20-carbon polyunsaturated fatty acids which are constituents of phospholipids found in the cell membrane of all mammalian tissues. Their structure is characterized by a cyclopentane ring with two aliphatic side chains. Thromboxanes have similar side chains but differ from the primary prostaglandins in having an oxane ring structure (see Figure 1) (4). Arachidonic acid is the main precursor in man and gives rise to thromboxane A_2 along with the other 2-series prostaglandins (PGE_2, $PGF_{2\alpha}$, PGD_2 and prostacyclin or PGI_2) (5). Dihomo-y-linolenic acid which is a trienoic analog of arachidonic acid, gives rise to the 1-series and is present at much smaller concentrations. Eicosapentaenoic acid, precursor of the 3-series, is normally absent in the body (6, 7). Thromboxane A_2 aggregates platelets and is a powerful vasoconstrictor (2). It has been shown to be produced in platelets, spleen, lung, polymorphonuclear leucocytes, brain, and inflammatory granuloma (8).

Figure 1. Basic Structure of the (1) Prostaglandins Which are Differentiated by their Different Cyclopentane Substituents, and (2) Thromboxanes (4).

1



2



The synthesis of thromboxane A_2 from arachidonic acid is shown in Figure 2 (9). Arachidonic acid is esterified and fixed mainly in membrane phospholipid. It must be liberated from the phospholipid before it can be metabolized by oxidative tissue enzymes. This liberation is thought to be accomplished by phospholipase A_2. In platelets, phospholipase A_2 requires calcium for its biochemical activity which could act as a regulating mechanism (10). The activation of phospholipase A_2 occurs through a chemical or physical stimulation of the cell membrane (11). Arachidonic acid is then released from the second position of phospholipid molecules, principally phosphatidyl choline and phosphatidyl inositol (9). Several proposed inhibitors of phospholipase A_2 are glucocorticosteroids, steroid anti-inflammatory drugs, and Mepacrine (10).

There are several pathways for the metabolism of arachidonic acid once it is released from the phospholipid molecule in platelets and lung homogenates (10).

It may be the substrate for the animal lipoxygenase synthesis of 12-hydroperoxyarachidonic acid (HPETE) and its hydroxyacid (HETE). The physiological significance of this is unknown, but HPETE has been claimed to inhibit TXA_2 synthetase.

There may also be a spontaneous auto-oxidation to 15-hydro-peroxyarachidonic acid (15-HPAA).

Of primary importance to this paper is the cyclization of arachidonic acid and inclusion of molecular oxygen to form the unstable intermediate prostaglandin endoperoxides PGG_2 and PGH_2. The enzyme responsible for this action is cyclo-oxygenase which is present in the plasma membrane or endoplasmic reticulum (12).

PGH_2 is further metabolized in platelets by thromboxane synthetase to

Figure 2. Synthesis of Thromboxane A_2 and B_2 from Arachidonic Acid (9).

Phospholipids

↓ Phospholipase A_2

Arachidonic Acid

↓ Cyclo-oxygenase

Prostaglandin G_2

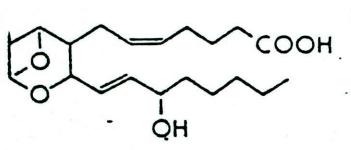
↓

Prostaglandin H_2

Platelets — Vascular Tissue

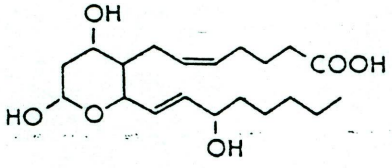
Platelets:

↓ Thromboxane Synthetase



Thromboxane A_2

↓ H_2O



Thromboxane B_2

Vascular Tissue:

↓ Prostacyclin Synthetase

Prostacyclin (PGI_2)

↓ H_2O

6-Keto-$PGF_{1\alpha}$

TXA_2 which is spontaneously hydrolyzed to TXB_2. In vascular tissue it is converted by prostacyclin synthetase to prostacylin (PGI_2) and its metabolite 6-keto-$PGF_{1\alpha}$ (Figure 2). PGH_2 is also converted to the stable primary prostaglandins PGE_2, $PGF_{2\alpha}$, and PGD_2. This occurs either nonenzymatically or through isomerase or reductase enzymes (12).

12L-hydroxy-5, 8, 10-heptadecatrienoic acid (HHT) and Malondialdehyde (MDA) are also metabolites of the prostaglandin endoperoxides (10).

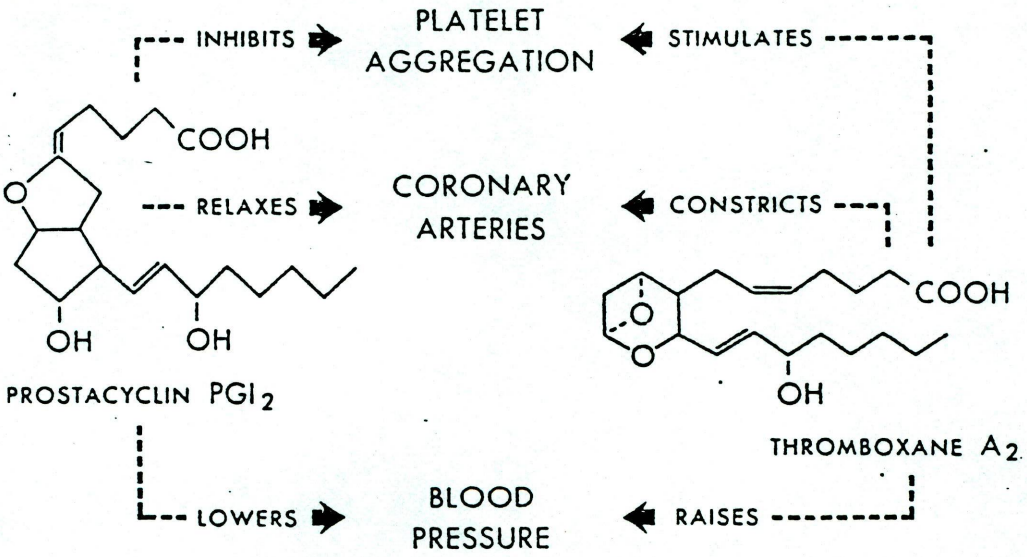
There are several known inhibitors of cyclo-oxygenase. They are: (1) aspirin, which has an irreversible action and persists as long as platelet life, (2) indomethacin, (3) phenylbutazone, (4) fenoprofen, and (5) sulfinpyrazone. The effects of these last four are lost when they or their metabolites are removed from the circulation (9).

Role of Thromboxane A_2 in Platelet Aggregation

Thromboxane A_2 as referred to earlier is a proaggregatory, vasoconstricting substance synthesized in platelets (13, 14). Prostacyclin formed in the blood vessel wall has opposite effects. It is a powerful anti-aggregating and vasodilating substance (10). The opposing effects of these two products of arachidonic acid metabolism is shown in Figure 3 (15).

Their physiological effects are mediated through the regulation of c-AMP levels. It is proposed that prostacyclin stimulates platelet adenylate cyclase, inhibits calcium mobilization, and thus prevents platelet aggregation. Thromboxane A_2 lowers c-AMP levels in platelets, stimulates calcium mobilization and, therefore, leads to platelet aggregation (10). A balance between TXA_2 and prostacyclin provides a homeostatic mechanism between platelets and the vessel wall to control platelet aggregation.

Figure 3. The Opposing Effects of Prostacyclin and Thromboxane A_2 (15).


INHIBITS
PLATELET AGGREGATION
STIMULATES
COOH
RELAXES
CORONARY ARTERIES
CONSTRICTS
OH
OH
PROSTACYCLIN PGI2
COOH
OH
THROMBOXANE A2
LOWERS
BLOOD PRESSURE
RAISES

An interaction between platelets and the vessel wall seems to occur. When platelets become in close contact with the vessel wall, they release their own prostaglandin endoperoxides which can then be used by the endothelial cells to synthesize prostacyclin. Platelets are then dispersed under the action of prostacyclin and further aggregation leading to thrombus formation will be prevented (5).

When the endothelial wall is damaged, platelets tend to clump and adhere to the vessel wall. Platelets undergo shape change and secrete the contents of their granules including TXA_2, promoting platelet aggregation and thrombus formation. Platelet contact with a healthy wall does not stimulate this clumping (11).

It is clearly seen that an upset in the balance between TXA_2 and prostacyclin would lead to increased platelet aggregation, thrombus formation, and possibly atherosclerosis. Other factors related to atherosclerosis such as high cholesterol, cigarette smoking, and diabetes have also been related to increased platelet aggregability (16-18). Nicotine seems to have a selective inhibitory effect on prostacyclin which may explain the increased stickiness of platelets reported in smokers (19).

Dietary Factors

A high polyunsaturated fatty acid diet has been shown to significantly reduce platelet aggregation (20) and produce changes in platelet function tests suggesting decreased platelet activation (21). Linoleic acid is also found to decrease arterial thrombus formation (22). Thus, a relationship between the synthesis of prostaglandins and diet is thought to occur.

Synthesis of prostaglandins may be depressed by linoleic acid. A high

intake of this fatty acid in animals results in its accumulation in platelet phospholipids, replacing arachidonic acid which is a precursor for prostaglandins (6). In this way, prostaglandin synthesis is inhibited. Conversion of linoleate to arachidonate may be enhanced in certain tissues when dietary linoleate is low (7).

Eicosapentaenoic acid (EPA) has been regarded as a possible therapeutic agent in prevention of atherosclerosis. Eskimos in Greenland have been shown to have a high content of EPA in the blood which probably originates from the high content of EPA in the diet. These Eskimos have a delayed atherosclerotic process and an increased tendency to bleed (23). Dyerberg, et al. suggests that EPA forms PGI_3 and TXA_3, prostaglandins of the 3-series. Since EPA has not been found to be a pro-aggregating agent, it is proposed that TXA_3 is inactive while PGI_3 is a potent anti-aggregating agent. This would shift the balance toward an anti-aggregating state (23). However, Hornstra observed that prostaglandins of the 3-series were hardly formed in a cod-liver oil diet (high-EPA) given to rats. He suggests the low thrombogenicity is due to depressed TXA_2 and PGI_2 production and possibly more of a decrease in platelet TXA_2 production pushing the balance toward an anti-thrombogenic state (24).

In a recent study, a diet rich in EPA was found to increase bleeding time and depress aggregation. Acetylsalicylic acid (ASA) given with the diet prolonged bleeding time longer than did ASA and the diet separately. The authors conclude that EPA in the diet affects the interaction between platelets and vessel walls other than altering prostaglandin synthesis (25).

Linolenic acid which can be converted to EPA in some species (22) may also be important. According to Lanzola, the ratio of linoleic to linolenic acid is related to their physiological effects (26).

High levels of linoleic acid in the diet may decrease TXB_2 production in platelets. Galli and his associates have shown a decreased production of TXB_2 in platelets of rabbits fed corn oil as compared to that produced in platelets of butter-fed animals. Subsequent reduction of the sensitivity of linoleic acid rich platelets to the aggregating action of arachidonic acid was observed (27). A diet high in polyunsaturated fatty acids may be beneficial to persons with atherosclerotic heart disease because of its anti-aggregating and TXB_2 lowering ability. The purpose of the present study is to measure changes in TXB_2 production in human subjects on a low fat, high polyunsaturated fatty acid diet.

METHODOLOGY

Subjects

Fourteen men between the ages of 32 and 76 volunteered to participate in this research project. Names were obtained from the cardiac rehabilitation program at Loma Linda Universty and through advertising in the local area. Criteria for selection were serum cholesterol above 220 and/or serum triglycerides above 135 or if they had experienced a heart attack. The subjects were fed three meals a day for twenty-eight days. Breakfast and supper were served in the Department of Nutrition research kitchen and a sack lunch was provided for the noon meal.

Diet

A ten-day cycle lacto-ovo-vegetarian menu was designed for this project (see Appendix). The meals met the RDA for all nutrients and had 20-25% calories supplied by fat, 14-17% calories supplied by protein, and approximately 60-65% calories supplied by carbohydrate. The mean P/S ratio of the diet was 3.6, and the mean calorie level was 1,952. Sodium content was kept at five grams or less per day (see Appendix for complete breakdown of each day's nutrients relevant to this study). The menu was designed to include foods typical of the American diet but with appropriate substitutions to lower fat and cholesterol content. For example, skim milk, egg substitute, low fat, low cholesterol cheese, and fat free salad dressing was used. The main source of fat was liquid safflower oil which was added to food in cooking since no visible fats were allowed.

Subjects ate only the food given them. They were instructed not to smoke or consume alcohol during the study period. Herb tea and cereal beverages were unlimited. Subjects were also instructed not to take aspirin during the

study period since it has been shown to inhibit prostaglandin synthesis (28).

Thromboxane Determination

A fasting blood sample was drawn into tubes containing EDTA. Blood was drawn the first day, at two weeks, and at the end of the study period. Breakfast was served immediately after blood drawing.

The blood sample was centrifuged and the plasma separated and frozen. The plasma samples were analyzed for thromboxane B_2 by radioimmunoassay as described by Granstrom (29).

The following reagents were used:

1. Assay Buffer. This buffer consists of 50 μM phosphate, 0.1% gelatin and 0.01% thimerosal, adjusted to pH 7.3.

2. Plasma Buffer. Human serum or plasma diluted 1:1 with phosphate buffer.

3. Phosphate Buffer. Consists of 6.8 g KH_2PO_4, 1.0 g gelatin, 0.1 g thimerosal, diluted to one liter with distilled water and adjusted to pH 7.3.

4. Thromboxane B_2 Standard. Purchased from the Upjohn Company, Kalamazoo, Michigan and diluted to 100 ng/ml.

5. Radiolabeled Thromboxane B_2. ^3H-TXB_2 tracer was purchased from New England Nuclear, Boston, Massachusetts. The 0.025 mCi/.25 ml Ethanol (125.0 Ci/m M) was added to 2.5 ml Ethanol and 100 μl of that was diluted to 10 ml with phosphate buffer.

6. Antiserum to Thromboxane B_2. Rabbit antiserum to TXB_2 was purchased from Seragen, Inc., Boston, Massachusetts.

7. Polyethylene Glycol (PEG). A 25% w/v (250 g/liter PEG) was prepared in distilled water and used to separate the antibody bound and free fractions.

8. Scintillation Fluid. The scintillation fluid used was Fred Snyder's modified Bray's solution (30).

All tests were run in duplicate in 12 x 75 mm polypropylene test tubes according to the protocol in Table 1. The samples were run in 50 μl and 100 μl dilutions. Six standard dilutions (5, 10, 25, 50, 100, and 250 pg/ml) were prepared and run before and after each set of samples. A plasma sample with a known amount of TXB_2 was used as a quality control (QC-1).

After addition of the antisera, the tubes were incubated for 16 hours at 4°C. At the end of the incubation, tubes were placed in an ice bath. Plasma buffer and PEG were added to each tube and vortex mixed vigorously for 5-10 seconds. The tubes were centrifuged immediately at 4°C for 30 minutes at 3400 rpm (1500 x g). After centrifugation, the supernatants were decanted and the precipitants redissolved in 1 ml of distilled water. The tubes were placed in a warm water bath and vortex mixed intermittently until the precipitate was completely dissolved. The resuspended precipitate was then transferred to polypropylene scintillation vials and counted with 10 mlmodified Bray's for 10 minutes.

Concentration of TXB_2 in the samples is determined from the standard curve. Calculations are as follows:

1. Counts per minute (cpm) for each set of duplicates are averaged.

2. Net counts for all samples are determined by subtracting from each the average plasma blank counts. Net counts for all standards are determined by subtracting from each the average buffer blank counts.

3. To determine the percent bound (% B) for each standard and sample, divide the average net cpm by the average net cpm of the total counts tubes.

Table 1. Protocol for Radioimmunoassay. (All Volumes are in ul Quantities.)

	Assay Buffer	Standard	Samples	Tracer	Antibody	Plasma Buffer	PEG
Total Counts	900	–	–	100	–	–	–
Buffer Blank	400	–	–	100	–	100	600
Plasma Blank	300	–	100	100	–	100	600
"0" Standard	300	–	–	100	100	100	600
Standards	200	100	–	100	100	100	600
Samples	200	–	100	100	100	100	600
QC-1	200	–	100	100	100	100	600

Added after the 16 hr incubation period

4. Calculate the normalized percent bound (% B/B_o) for each standard and sample as follows:

$$\% B/B_o = \frac{\% \text{ B of Standard or Sample}}{\% \text{ B of "o" Standard}} \times 100\%$$

5. Using semi-logarithmic graph paper, plot % B/B_o for each standard versus the corresponding picograms (pg) TXB_2 added.

6. Determine the pg TXB_2 in each sample by interpolation from the standard curve.

Total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides were determined by the Faculty Medical Staff Lab.

RESULTS AND DISCUSSION

Fourteen male subjects were given a 20-25 Calories % fat, high polyunsaturated fatty acid diet for 28 days. All subjects completed the study except for subject #13 who dropped out after the second week due to illness. Plasma samples were collected and thromboxane B_2 levels were measured by radioimmunoassay. Paired t-tests were used to analyze the data.

A 20-25 Calories % fat, high polyunsaturated fatty acid diet significantly increased plasma TXB_2 levels (Tables 2 and 3). A rise in TXB_2 was observed between initial values and those at two weeks with a decrease between weeks two and four. However, TXB_2 was still significantly elevated at the fourth week when compared with the initial values.

These results differ with previous data presented by Galli, et al. indicating that TXB_2 synthesis is depressed on a high linoleic acid diet (27). Linoleic acid is also known to replace arachidonic acid in platelet phospholipids implying an inhibition of prostaglandin and thromboxane synthesis (6).

It is difficult to explain our results in light of the current research done thus far. The inconsistent pattern observed may be due in part to the instability of TXB_2 under storage conditions. McCann, et al. report that prolonged storage of TXB_2 for one month or more at -20°C was unsatisfactory. Initial values of TXB_2 compared with those at one, two, three and four months of storage showed predominantly losses of TXB_2, but occasionally there were marked increases (31). Initially, we were unaware of this storage problem and our plasma samples were frozen for three months before analysis was done.

Plasma levels of 6-keto-$PGF_{1\alpha}$ (stable metabolite of prostacyclin) were also measured and reported by Violet Komm, Loma Linda University Masters Thesis, 1982. No significant changes were observed over the four week-period (Table 2).

Table 2. Mean Change and Standard Error of Plasma TXB_2, 6-keto-$PGF_{1\alpha}$ and Serum Lipid Levels.

	0-2 Weeks	2-4 Weeks[a]	0-4 Weeks	4-8 Weeks[b]
TXB_2 pg/ml	99 ± 30 ($p < .01$)[c]	-88 ± 34 ($p < .05$)	15 ± 6 ($p < .05$)	
Total Cholesterol mg/dl	-45 ± 6 ($p < .001$)	-6 ± 4 (N.S.)	-49 ± 7 ($p < .001$)	45 ± 16 ($p < .025$)
HDL Cholesterol mg/dl	-9 ± 3 ($p < .05$)	1 ± 2 (N.S.)	-8 ± 3 ($p < .05$)	7 ± 3 ($p < .025$)
LDL Cholesterol mg/dl	-27 ± 6 ($p = .001$)	-12 ± 4 ($p < .025$)	-41 ± 8 ($p < .001$)	29 ± 19 (N.S.)
Triglyceride mg/dl	-106 ± 96 (N.S.)	22 ± 14 (N.S.)	-84 ± 103 (N.S.)	38 ± 65 (N.S.)
6-keto-$PGF_{1\alpha}$[d] pg/ml	101 ± 58 (N.S.)	-7 ± 35 (N.S.)	53 ± 37 (N.S.)	

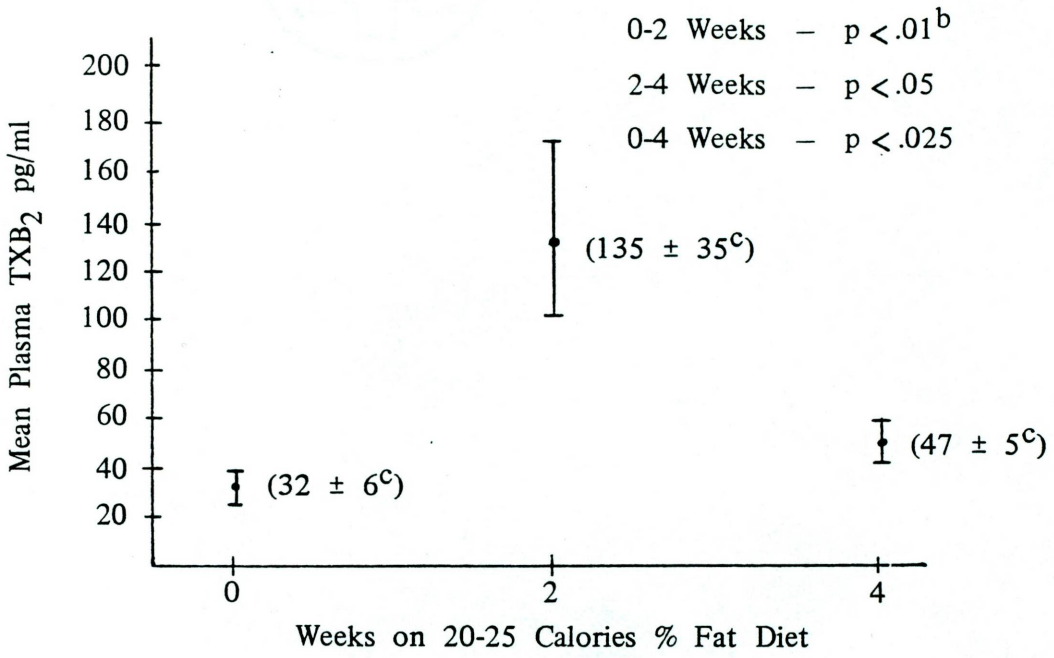
[a]When comparing values at 4 weeks means used included only the 13 subjects who completed the study.

[b]Only serum lipids were measured at 8 weeks.

[c]Null hypothesis: Change is not significantly different from zero.

[d]Data used is from Violet Komm, Loma Linda University Masters Thesis March 1982.

Table 3. Mean Plasma TXB_2 Levels in 14 Human Subjects.[a]



[a]Means do not include data from subject No. 13.

[b]Null hypothesis: Change is not significantly different from zero.

[c]Standard error.

Changes in serum lipids were also measured (Table 2). Serum cholesterol levels were significantly decreased by the low 20-25 Calories % fat, high polyunsaturated fatty acid diet. Most of the decrease in serum cholesterol took place during the first two weeks with a small insignificant decrease occurring during the final two weeks. The cholesterol lowering effect of this diet may be beneficial in preventing coronary heart disease. Miettinen, et al. found that a cholesterol-lowering diet was associated with a significant decrease in mortality from coronary heart disease in men (32).

Measurement of serum cholesterol four weeks after the end of the study period showed a significant increase back toward the baseline values. Both HDL and LDL cholesterol were significantly reduced over the four-week period. Measurement of these variables four weeks after the end of the study period showed a tendency to increase, but this increase was not significant. No significant changes were observed in serum triglyceride levels.

Blood lipid levels appear to be related to the metabolism of prostaglandins. High serum cholesterol levels have been related to an increased production of TXB_2 in isolated platelets (33, 34). High levels of triglycerides may be related to a decrease in prostacyclin levels in the serum (35). Several significant correlations which were observed between TXB_2, 6-keto-$PGF_{1\alpha}$, and serum lipids are reported in Table 4.

Table 4. Correlations Between TXB_2, 6-keto-$PGF_{1\alpha}$ and Serum Lipids.[a]

	TXB_2	Total Cholesterol	HDL Cholesterol	LDL Cholesterol	Triglycerides
6-keto-$PGF_{1\alpha}$	.5636 ($p < .05$)[b]	N.S.	-.6057 ($p < .05$)	N.S.	.6832 ($p < .01$)
TXB_2	–	N.S.	-.5085 ($p < .05$)	N.S.	.5739 ($p < .05$)

[a]Pearson correlation coefficient is used.

[b]Null hypothesis: Correlation is not significantly different from zero.

SUMMARY

Fourteen male subjects with elevated lipid levels were given a low (20-25 Calories %) fat, high polyunsaturated fatty acid diet for 28 days. Fasting blood samples were drawn at zero, two, and four weeks and analyzed for plasma TXB_2 and serum lipids. Plasma TXB_2 was significantly increased the first two weeks with a subsequent decrease the final two weeks. Values were still significantly elevated at four weeks when compared with initial values. Serum cholesterol, HDL cholesterol, and LDL cholesterol were significantly decreased over the four-week period. No significant changes were observed in serum triglycerides.

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APPENDIX

Table 5. Age and Weights of 14 Human Subjects on a 20 - 25 Calories % Fat Diet for 28 Days.

Subject #	Age	Weight (lbs.)
1	57	158
2	60	142
3	50	191
4	57	204
5	41	202
6	48	215
7	76	142
8	45	232
9	60	206
10	51	196
11	32	178
12	62	174
13	36	178
14	63	165
Mean	53 ± 12[a]	184 ± 27[a]

[a] Standard deviation.

Table 6. Plasma TXB_2 Levels (pg/ml) in 14 Human Subjects.

Subject #	0 Weeks	2 Weeks	4 Weeks
1	44	44	72
2	33	60	52
3	23	113	82
4	50	82	34
5	32	48	42
6	16	78	52
7	20	170	44
8	88	512	72
9	19	42	18
10	31	156	37
11	11	60	24
12	43	230	37
13[a]	185	220	-
14	5	170	47
Mean	43± 46[b]	142± 125[b]	47± 19[b]

[a] Subject # 13 dropped out of study after second week due to illness.

[b] Standard deviation.

Table 7. Serum Cholesterol Levels (mg/dl) in 14 Human Subjects.

Subject #	0 Weeks	2 Weeks	4 Weeks	8 Weeks
1	209	194	196	209
2	224	152	151	194
3	306	247	242	220
4	244	182	173	276
5	193	158	143	302
6	240	185	184	214
7	192	163	146	173
8	224	212	187	146
9	256	223	193	231
10	285	230	217	225
11	228	186	200	267
12	245	176	168	310
13[a]	334	260	-	-
14	258	238	265	284
Mean	246± 41[b]	200± 35[b]	190± 36[b]	235± 50[b]

[a] Subject # 13 dropped out of study after second week due to illness.

[b] Standard deviation.

Table 8. HDL Cholesterol Levels (mg/dl) in 14 Human Subjects.

Subject #	0 Weeks	2 Weeks	4 Weeks	8 Weeks
1	47	49	55	59
2	40	29	29	47
3	86	47	44	51
4	23	19	26	36
5	38	25	33	54
6	30	23	20	38
7	42	39	29	38
8	45	38	28	23
9	36	31	37	25
10	50	42	41	47
11	37	26	40	27
12	42	44	39	65
13[a]	29	28	-	-
14	69	52	61	62
Mean	44± 16[b]	35± 11[b]	37± 12[b]	44± 14[b]

[a] Subject # 13 dropped out of study after second week due to illness.

[b] Standard deviation.

Table 9. LDL Cholesterol Levels (mg/dl) in 14 Human Subjects.

Subject #	0 Weeks	2 Weeks	4 Weeks	8 Weeks
1	143	133	128	138
2	169	106	102	130
3	200	152	112	149
4[a]	-	114	102	225
5	92	84	64	195
6	165	110	111	96
7	132	101	91	96
8	118	111	90	96
9	196	164	128	163
10	181	156	145	104
11	158	124	115	181
12	148	113	107	209
13[b]	214	214	-	-
14	168	167	184	72
Mean	160± 34[c]	132± 35[c]	114± 29[c]	143± 49[c]

[a] LDL Cholesterol could not be computed at 0 weeks because of high triglyceride value.

[b] Subject # 13 dropped out of study after second week due to illness.

[c] Standard deviation.

Table 10. Serum Triglyceride Levels (mg/dl) in 14 Human Subjects.

Subject #	0 Weeks	2 Weeks	4 Weeks	8 Weeks
1	97	58	63	62
2	74	83	101	85
3	100	242	430	100
4	1480	247	227	73
5	316	243	228	265
6	223	260	265	298
7	88	113	131	196
8	303	315	345	133
9	118	139	141	215
10	272	159	155	369
11	163	178	226	294
12	276	96	112	178
13[a]	456	-	-	-
14	104	96	98	750
Mean	291± 361[b]	171± 82[b]	194± 107[b]	232± 183[b]

[a] Subject # 13 dropped out of study after second week due to illness.

[b] Standard deviation.

Table 11. Plasma 6-Keto-$PGF_{1\alpha}$ (pg/ml) in 14 Human Subjects[c]

Subject #	0 Weeks	2 Weeks	4 Weeks
1	300	140	145
2	145	170	220
3	110	250	290
4	410	140	180
5	140	370	210
6	150	450	300
7	140	210	300
8	210	350	280
9	215	250	200
10	330	320	320
11	250	500	280
12	160	170	260
13[a]	220	860	190
14	120	130	380
Mean	207± 89[b]	308± 198[b]	259± 65[b]

[a] Subject # 13 dropped out of study after second week due to illness.

[b] Standard deviation.

[c] Data used is from Violet Komm, Loma Linda University Masters Thesis March 1982.

Table 12. Ten-day Lacto-ovo-vegetarian Menu

DAY 1

Breakfast

8 oz. skim milk
1 blueberry muffin
3/4 c. dry cereal
8 oz. orange juice or 6 oz. apple juice
1/2 large banana

Lunch

Sandwich:
2 slices whole wheat bread, 2 tbsp. turkey spread, lettuce leaf
1 c. coleslaw + 1 tbsp. oil*
1 nectarine
6 oz. can V-8 juice

Dinner

1 c. spaghetti with 3/4 c. sauce with 1 tsp. oil
3 tbsp. Parmesan cheese
1/2 c. spinach with lemon wedge
Lettuce salad with radishes
Zero calorie dressing
1 slice hot garlic bread
1 peachy shake
1 c. diced watermelon

*All oil used is liquid safflower oil.

DAY 2

Breakfast

1 c. skim milk
1/2 English muffin
1 tbsp. jelly
10 cottage fries + 1 tsp. oil
3/4 c. dry cereal
8 oz. orange juice or 4 oz. grape juice
1 medium peach

Lunch

Sandwich:
1 hot dog bun, 1 Loma Linda linkett
with mustard and relish
2 carrot sticks, 2 celery sticks,
3 broccoli flowers, 3 cauliflowers
Dressing + 2 tbsp. oil
1 orange
1 small can apple juice

Dinner

2 c. sukiaki
1 c. rice
1 fortune cookie with tea
Boysenberry milk shake
1/2 c. frozen fruit medley

DAY 3

Breakfast

1 c. skim milk
Fruit toast
2 slices toast
3/4 c. dry cereal
8 oz. orange juice

Lunch

Sandwich:
2 slices bread, 1 1/8" slice bologna + 1 tsp. oil
2 slices tomato, 1 leaf lettuce
Garden vegetable soup
8 oz. lemonade
1 small banana

Dinner

1 stuffed bell pepper
1/2 c. frozen baby carrots
Lettuce wedge with Zero dressing
1 slice bread
1 tbsp. jelly
1 1/3 c. fruit juice medley
8 oz. fudge pudding

DAY 4

Breakfast

1 c. skim milk
1 c. oatmeal + 1 tsp. oil
2 tbsp. raisins
1 slice toast
1 tbsp. jelly
2 fresh pear halves
8 oz. orange juice or grapefruit juice

Lunch

1 vegeburger
1 hamburger bun with yogurt, sliced tomato, mustard, lettuce, pickles
1 c. carrot and raisin salad
1 small can grapefruit juice

Dinner

Vegetarian pot pie
1 c. Italian mixed vegetables
1/2 c. parsley potatoes
4 small radish roses
1/2 slice bread
1 tbsp. jelly
Raspberry delight shake
1 peach

DAY 5

Breakfast

1 c. skim milk
3 4" pancakes + 1 tsp. oil
1 c. hot cinnamon applesauce
8 oz. orange juice or 6 oz. pineapple juice

Lunch

1 c. French onion soup
1 slice French bread
1/2 c. potato salad
3 slices tomatoes
1 small fresh pear
4 oz. bottle grape juice

Dinner

Tostadas--get 2 cups vegetables (lettuce, tomato, etc.)
2 tbsp. low-fat yogurt
1 tbsp. hot sauce
1/2 c. Mexican rice
1/2 c. Mexican wax beans
Strawberry banana shake
1 nectarine

DAY 6

Breakfast

8 oz. skim milk
1/2 English muffin
1 tbsp. jelly
3/4 c. dry cereal
10 cottage fries + 2 tsp. oil
8 oz. orange juice or 4 oz. grape juice
1 peach

Lunch

Stew
1/2 slice French bread
2 apricots
8 oz. lemonade

Dinner

2 lasagna swirls
1/2 c. zuchinni italiano
1 slice Italian bread with garlic
1 c. skim milk
Lettuce salad with radishes and low calorie dressing
Melon medley: 1 c. watermelon, 1/8 c. honeydew, 1/4 c. cantaloupe

DAY 7

Breakfast

1 c. skim milk
1 c. oatmeal + 2 tsp. oil
2 tbsp. raisins
1 slice whole wheat toast
1 tbsp. jelly
8 oz. orange juice or grapefruit juice
1 orange

Lunch

Sandwich:
2 slices bread, 2 T. egg salad
3 carrot sticks, 3 celery sticks 3" long
1 small can apple juice
1 nectarine

Dinner

2 enchiladas
1/2 c. Mexican rice
1/2 c. Mexican green beans
Orange juice/milk cooler
2 plums

DAY 8

Breakfast

1 c. skim milk
1 blueberry muffin
3/4 c. dry cereal
8 oz. orange juice or 2/3 c. apple juice
1/2 large banana

Lunch

Fresh vegetable soup
2 soda crackers
1 slice low-fat cheese (1 oz.)
1 pear
1 small can pineapple juice

Dinner

1 1/3 c. low-calorie oriental + 1 tbsp. soy sauce
3/4 c. rice + 1 tsp. oil
1 c. oriental vegetables
2 fortune cookies + tea
1 c. skim milk
Fruit kabobs--pineapple, oranges, apples, bananas

DAY 9

Breakfast

1 c. skim milk
Fruit toast
2 slices toast
3/4 c. dry cereal
8 oz. orange juice

Lunch

Sandwich:
2 slices bread, 2 tbsp. cheese spread
3 slices tomatoes, lettuce leaf
3 broccoli flowers, 3 cauliflowers
Zero dressing
4 oz. bottle grape juice
Dried fruit medley:
2 dried apricot halves, 1 dried pear half,
1 dried apple ring

Dinner

Split pea and cottage cheese loaf
3/4 c. steamed broccoli
3/4 c. stewed tomatoes + 1/2 tsp. oil
1/2 baked potato with 1/4 c. grated cheese
Mock sour cream
1 slice whole wheat bread
1 tbsp. jelly
1/2 cantaloupe with 8 strawberries
8 oz. skim milk

DAY 10

Breakfast

1 c. skim milk
3 4" pancakes + 1 tsp. oil
1 c. hot cinnamon applesauce
1 c. orange juice or 6 oz. pineapple juice

Lunch

Sandwich:
2 slices bread, 2 tbsp. sandwich spread
2 tomato slices, lettuce leaf
1 large apple
Nut and raisin cup (12 peanuts, 2 T. raisins)
1 small can tomato juice

Dinner

1 1/2 falafels with 1/2 c. garbanzos on each one
1 1/2 c. raw vegetables and 1/8 c. plain yogurt
1/2 c. tarragon carrots
Tropical shake
1/2 c. frozen blueberries

Table 13. Daily Composition of Nutrients in Ten Day Cycle Menu

Day	Calories	% Fat	% Protein	% Carbohydrate	Cholesterol(mg)	Sodium(mg)	P/S Ratio
1	2146	19.7	13.4	66.9	26	2823	3.57
2	2082	20.0	17.0	63.0	101	1820	2.95
3	1832	22.0	14.0	64.0	74	1898	2.82
4	1992	22.5	12.0	65.5	30	3048	3.28
5	1895	24.7	15.0	60.3	39	3244	3.39
6	1938	20.6	13.9	65.5	105	4435	1.85
7	1941	21.0	14.8	64.2	35	4009	3.76
8	1567	25.0	15.4	59.6	15	3150	4.78
9	1886	24.1	13.9	62.0	36	3471	3.26
10	2242	24.1	14.7	61.2	18	4374	5.80
Mean	1952	22.4	14.4	63.2	48	3227	3.55